

NEW DATA ON *TULOSTOMA SUBSQUAMOSUM* (*GASTEROMYCETES*)

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ABSTRACT — The presence of *Tulostoma subsquamosum* has been confirmed in Spain and by extension throughout Europe. Only a reduced number of records of this species were made in India, Pakistan and Argentina. A macroscopic and microscopic study of Spanish collections was carried out and compared with *T. subsquamosum* and *T. melanocyclus* holotypes. The most important features are illustrated by microphotographs and scanning electron microscopy.

KEY WORDS: chorology, taxonomy, *Tulostoma melanocyclus*, *T. subsquamosum*.

Since Long & Ahmad (1947) first described *Tulostoma subsquamosum* Long & S. Ahmad from material collected in India, very few references have been added for this species. Wright (1987) considered it a critical taxon and gave two more records: the first one from Pakistan (not far from the type locality) and the second from Argentina that notably expanded its distribution area. Later, Calonge & Wright (1989) recorded its presence in Spain with some doubts due to the great macroscopic similarity observed between their material and *T. melanocyclus* Bresadola. We have no further knowledge about any additional record of *T. subsquamosum*. For this reason, it is believed that material collected and studied by us during the last years in Spain, the macro- and microscopic features of which fits fairly well with those of the *T. subsquamosum* holotype, are especially interesting. Thus, we can confirm the presence of this fungus in Spain (and therefore in Europe), which not only extends notably its distribution area, but also contributes to a better knowledge of this critical taxon.

MATERIALS AND METHODS

The collections studied are from the herbaria of the following institutions: University of Alcalá de Henares, Madrid, Spain (AH); Real Jardín Botánico of Madrid, Spain (MA-Fungi); University of Buenos Aires, Argentina (BAFC); Royal Botanic Gardens, Kew, Surrey, England (K).



The colour of the gleba has been determined by comparison with the Natural Colour Guide of Locquin (1975), with the corresponding code in parentheses. Photographs, both of light microscope (LM) and scanning electron microscope (SEM), were made according to Moreno *et al.* (1995).

DESCRIPTION

Tulostoma subsquamosum Long & S.Ahmad, Farlowia 3:241, 1947.

Figs. 1-12, 17-24

Material studied. SPAIN: Almería: Sierra de María, 26-XII-1987, J.A.Oria de Rueda, MA-Fungi 20304. Cádiz: Grazalema, IV-1987, P.Thompson, MA-Fungi 20250 (as *T. melanocyclus*). Madrid: Meco, 26-XI-1989, A.Bernao, AH 13579. Navarra: Alsasua, I-V-1973, E.Arrondo, MA-Fungi 20290 (as *T. melanocyclus*) (doubtful). Toledo: km 41 ctra. Arganda-Mora de Toledo, 28-IV-1986, C.Gómez, MA-Fungi 16316 (as *T. melanocyclus*). Valencia: El Saler, 23-XI-1989, A.Burguete, AH 12449. Zaragoza: Bujaraloz, 4-X-1991, A.Altés *et al.*, AH 13826 and 13827; *ibidem*, 17-II-1995, K.Kreisel *et al.*, AH 18421; *ibidem*, 31-X-95, G.Moreno & A.Altés, AH 19020, 19021, 19022, 19023, 19024 y 19025; Pina de Ebro, 9-XII-1989, J.Blasco, MA-Fungi 32137 (as *T. melanocyclus*). INDIA: Gurdaspur, Jaggatpur, VIII-1938, S.Ahmad 258 (BAFC) HOLOTYPE. ARGENTINA: Chaco, Avia Teray, VII-1930, A.Castellanos, BAFC 29454 (ex BA 1245).

Material studied of other species. ITALIA: "In glavrosis prope Tridentum", 1902, G.Bresadola, K, HOLOTYPE of *T. melanocyclus*.

Spanish material of this species (16 collections) exhibits the following features: *Basidiocarps* 15-49 mm long, more frequent length 20-40 mm. *Spore-sac* 7-12,5 mm diam, globose to subglobose. *Mouth* tubular, more or less developed, normally small (0,3-1 mm diam) when compared with the spore-sac size, peristome concolorous with the endoperidium. *Exoperidium* seemingly granulose, more or less persistent, mixed with sand or soil particles, even though it tends to form a thin membrane in contact with the endoperidium, which in most basidiocarps persists as white patches. *Endoperidium* with numerous remains of exoperidium, though in naked zones it appears smooth and dirty white to pale ochre colored. *Gleba* ferruginous (rubeus A3f). *Stipe* 10-43 x 1-4 mm, cylindrical, rather straight, typically covered with dark brown scales, somewhat striate in the upper third, with a slightly wider bulbous base and often covered with mycelial remains.

Basidiospores 4,5-6(-6,5) µm diam, globose to subglobose, pale yellow, notably verrucose. Under SEM a more or less dense net of irregular crests with reticulate to subreticulate appearance can be observed (Figs. 17-21). *Capillitium* 3,5-11 µm diam, thick walled, often with a continuous lumen, branched, pale yellow, very to not at all broadened at the frequent septa which sometimes are ochraceous. *Exoperidium* formed by 4-8 µm diam, tortuose, thick-walled, branched, subhyaline to pale yellow hyphae, usually not broadened at the colourless septa, mixed with other thin walled and very frequently septate hyphae. The latter being easily disjointable and giving rise to cells with very diverse shapes and sizes (Fig. 23), similar to the sphaerocysts of exoperidium of *T. squamosum* Gmelin: Pers. (Moreno *et al.*, 1992). *Endoperidium* formed by hyphae similar to those of capillitium, 3-5 µm diam, compactly interwoven, thick walled, usually with a continuous lumen, branched, subhyaline, more or less broadened (up to 9 µm diam) at the usually pale ochre septa.

REMARKS

The holotype collection of *T. subsquamosum* actually consists of spore sacs and fragments of stipes belonging to three basidiocarps (Fig 1), although Long & Ahmad (1947) cited four specimens in the mentioned collection. The features we can observe in this material agree fairly well with the given description and show no differences when compared with those of the Spanish material. The presence of sphaerocyst-like cells in the exoperidium of the holotype is remarkable. This character had not been underlined until now in this species, and also not observed in the other studied material. Accordingly, it should be stressed that the presence of these sphaerocysts is not always simple to check. This difficulty may be due to the poor state of conservation of the exoperidium or to the fact that specimens were collected a long time after their complete development: furthermore the great quantity of sand and soil particles mixed with the exoperidium are very difficult to eliminate. Nevertheless, by choosing carefully the material it is possible to observe these cells in the white patches remaining on the endoperidium. So far, little attention was paid to the microscopy of the exoperidium and the endoperidium; however, it appears to be an interesting character within a genus not exhibiting so much good taxonomic features. This is the case in *T. squamosum* (Moreno *et al.*, 1992) and *T. cyclophorum* Lloyd (Wright, 1987; Moreno *et al.*, 1990), as well as in *T. subsquamosum*. We think it is necessary to pay more attention to these structures in any future taxonomic studies on the genus *Tulostoma*.

Another especially important feature in this species is the spore ornamentation. It consists of diversely sized and shaped crests, which form a more or less tangled usually rather dense subreticulate as shown by SEM (Figs. 2,3,17-22). Sometimes this net is looser and reminds of the typical spore ornamentation of *T. fimbriatum* Fr. However, confusion between both species is to be excluded since the latter has a fibrillose-fimbriate mouth and a clearly hyphal exoperidium. The sample MA-Fungi 20290 is the only one that shows a different spore ornamentation, not subreticulate but consisting of short crests with more or less conical spines. However, its other characters agree with *T. subsquamosum*, in particular the presence of sphaerocysts in the exoperidium (Fig. 23). For this reason we maintain this collection for the moment in *T. subsquamosum*.

The presence of a stipe almost completely covered with dark brown scales, a more or less developed tubular mouth, and a peristome concolorous with the endoperidium, are other important data to characterize this taxon.

T. squamosum shares many of the indicated features and is very similar to *T. subsquamosum*. The latter also has a tubular mouth, a stipe covered with dark brown scales, and spores notably ornamented under LM.

Therefore, it is not surprising that S. Ahmad had previously misinterpreted the holotype of *T. subsquamosum* as *T. squamosum*, even less if we realize that its distribution was at that time exclusively European. But later, when Long and S. Ahmad compared their material with typical specimens of *T. squamosum* from Hungary and France, they did not doubt that both species were different (Long & Ahmad, 1947). The basic differences between both species are that *T. squamosum* has much larger scales on the stipe, an exoperidium that tends to remain as circular patches over the endoperidium, and a different spore ornamentation with conical spines formed by several apically fused elements. However, as mentioned before, *T. squamosum* also has sphaerocysts in the exoperidium, and the spore ornamentation sometimes appears not so different from that of *T. subsquamosum* under SEM. These data strengthen the relation of similarity between both

species. Even so, excepting a few problematic cases, we believe that it should not be too difficult to distinguish both taxa.

In the specific case of Spain, and focusing on the material studied, *T. subsquamosum* has been repeatedly mistaken for *T. melanocyclus*. However, examination of holotype of the latter disclose enough differences between the two species. The macroscopic aspects of *T. melanocyclus* (Fig. 13) and *T. brumale* are almost identical, with gracile habit, small sporangium (usually not larger than 10 µm diam), tubular mouth (*T. melanocyclus*: Fig 14), reddish brown peristome, stipe with rather small scales, and mycelial remains at the bulbous base, but *T. melanocyclus* has a hyphal exoperidium and usually a darker stipe than *T. brumale*. SEM reveals the spore ornamentation consisting of conical spines formed by several apical united elements (Figs. 15,16), a feature shared with *T. squamosum*, the capillitium slightly broadened at the septa, and the lack of sphaerocysts in the exoperidium.

All Spanish samples so far referred to *T. melanocyclus* have features not fitting with the concept of this taxon and so have to be excluded from this species. This implies the elimination of *T. melanocyclus* from the Spanish *Tulostoma* catalogue, not discarding its future incorporation. The revision of these samples, and others mentioned before, confirms the presence of *T. subsquamosum* in Spain and Europe, but also reveals its good representation in this country. For the same reasons that *T. subsquamosum* has been confused with *T. melanocyclus* in Spain we suspect that this also happened in other areas, especially in Mediterranean countries. Thus it would be opportunate to restudy all the material cited under *T. melanocyclus* originating from that area.

Futhermore, examination of a specimen from Argentina allows us to confirm the presence of *T. subsquamosum* in the Southamerican subcontinent where so far only one reference was known (Wright, 1987). Even though the features of this collection agree with the given description, we must remark that the corresponding spores are slightly smaller and that their ornamentation is not so prominent (Fig. 22). On the other hand, the presence of sphaerocysts in its exoperidium is specially notable (Fig. 24).

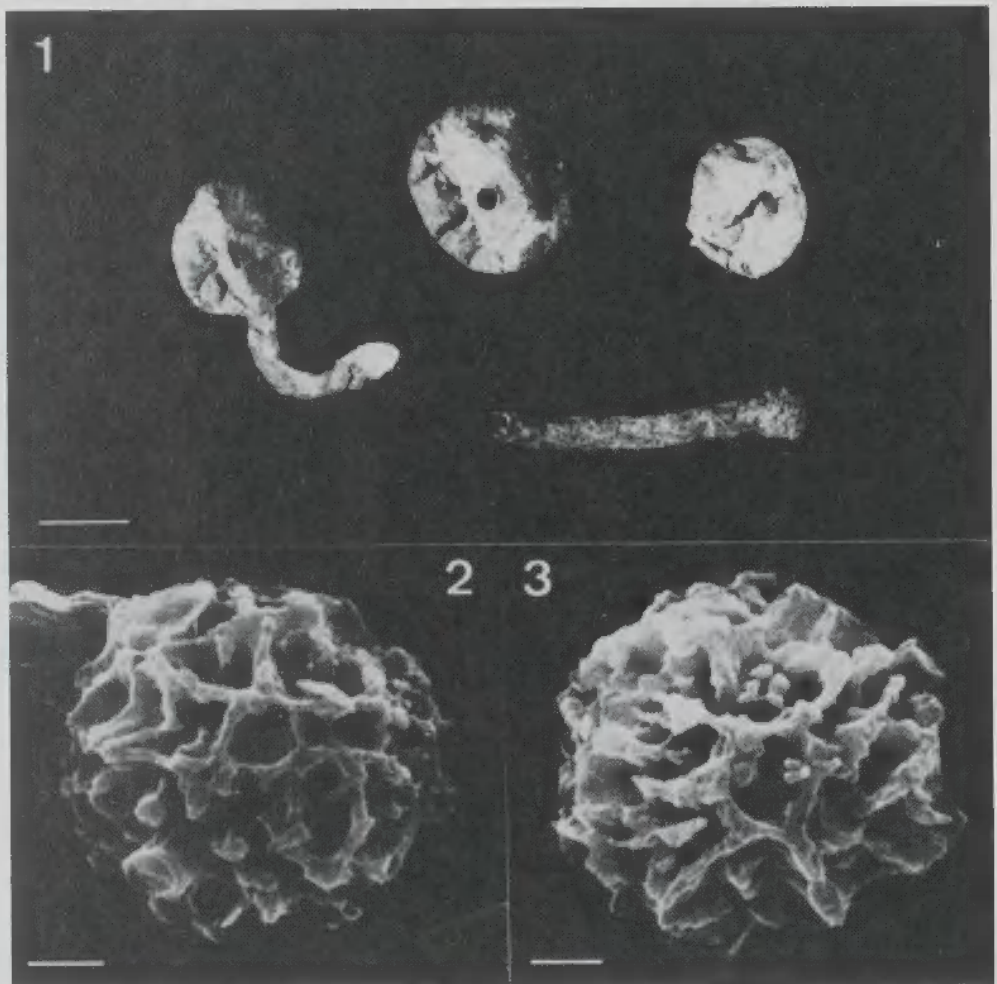
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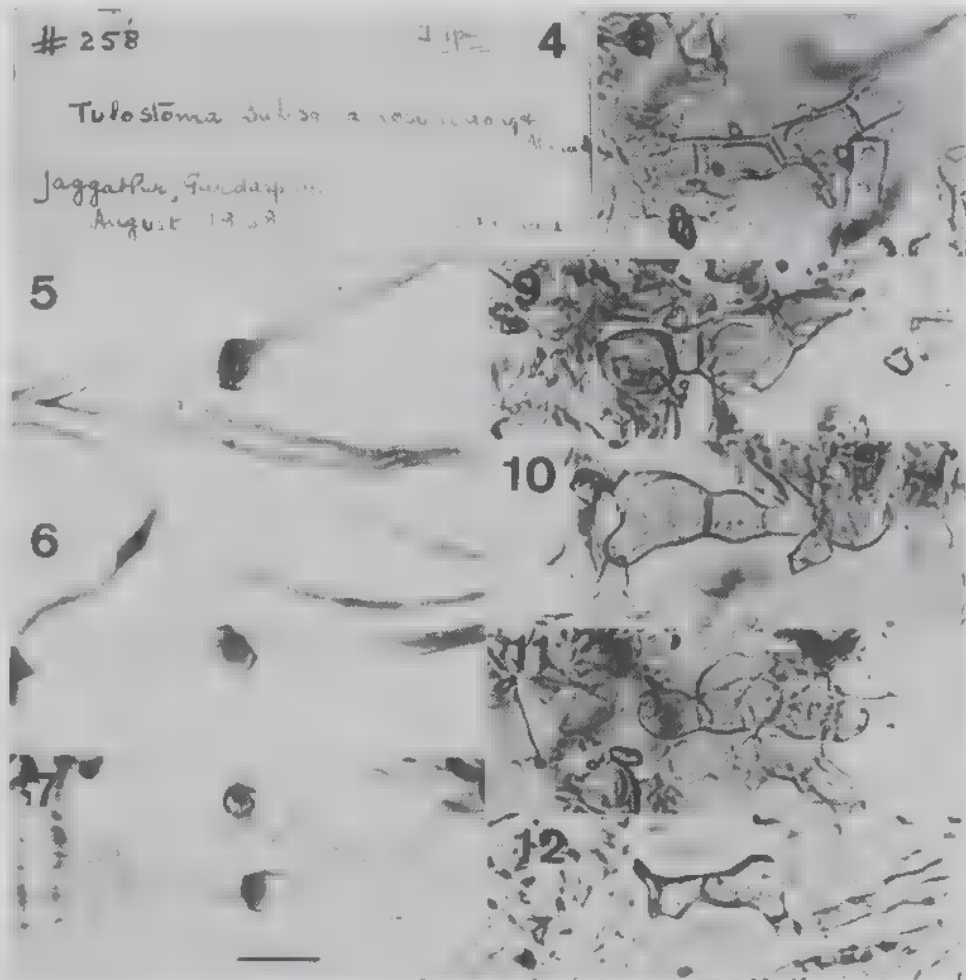
REFERENCES

- CALONGE F.D. & WRIGHT J.E., 1989 — El género *Tulostoma* Pers.:Pers. (*Gasteromycetes*) en España. *Boletín de la sociedad micológica de Madrid* 13: 119-137.
- LOCQUIN M.V., 1975 — *Natural Color Guide*. Publ. by the author, Sens, France. 12 pl.
- LONG W.H. & AHMAD S., 1947 — The genus *Tylostoma* in India. *Farlowia* 3: 225-267.
- MORENO G., ALTÉS A. & AYALA N., 1990 — *Tulostoma cyclophorum* Lloyd (*Gasteromycetes*, *Basidiomycotina*) nuevo para España peninsular. *Anales del instituto de biología de la universidad nacional de México, serie botánica* 60: 21-25.

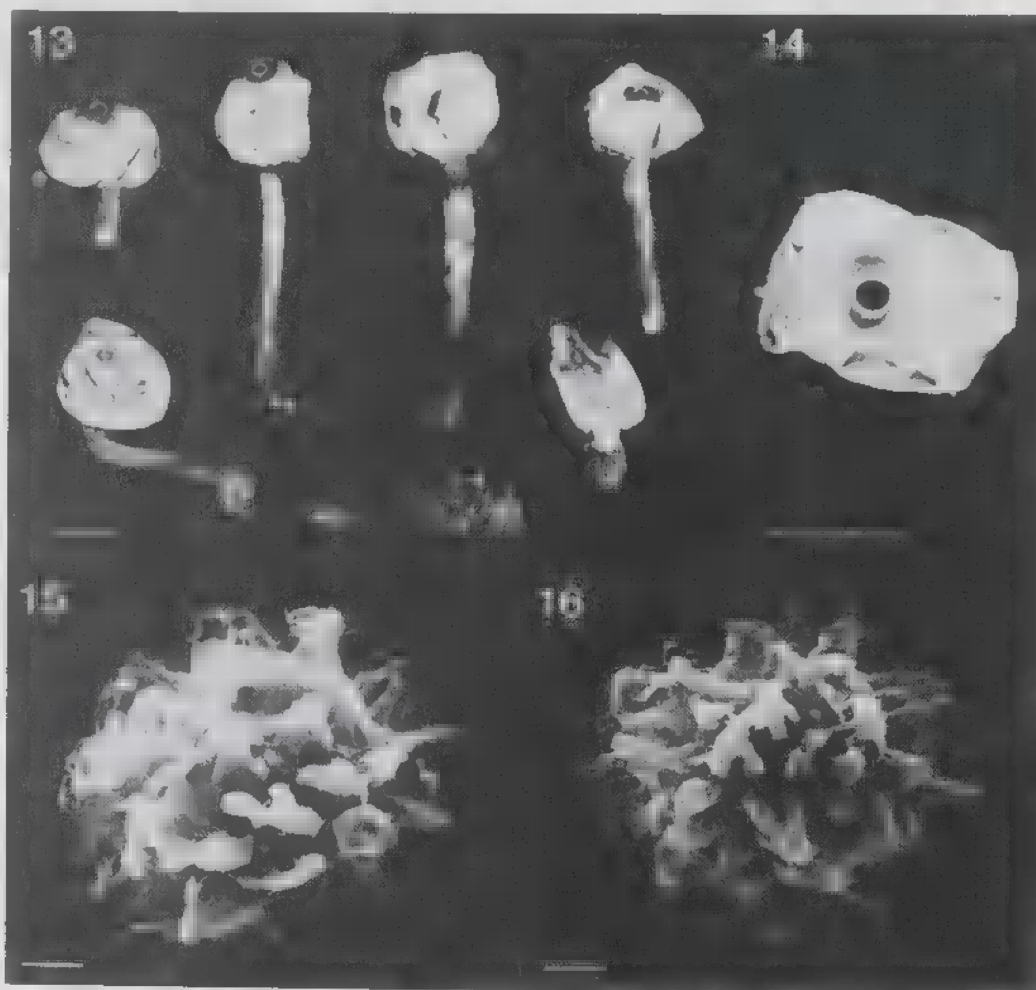
- MORENO G., ALTÉS A., OCHOA C. & WRIGHT J.E., 1995 — Contribution to the study of the *Tulostomataceae* in Baja California, Mexico. I. *Mycologia* 87: 96-120.
- MORENO G., ALTÉS A. & WRIGHT J.E., 1992 — *Tulostoma squamosum*, *T. verrucosum* and *T. mussooriense* are the same species. *Mycotaxon* 43: 61-68.
- WRIGHT J.E., 1987 — *The genus Tulostoma (Gasteromycetes). A world monograph.* J.Cramer, Berlin, Stuttgart, Germany. 338 pp., 50 pl.



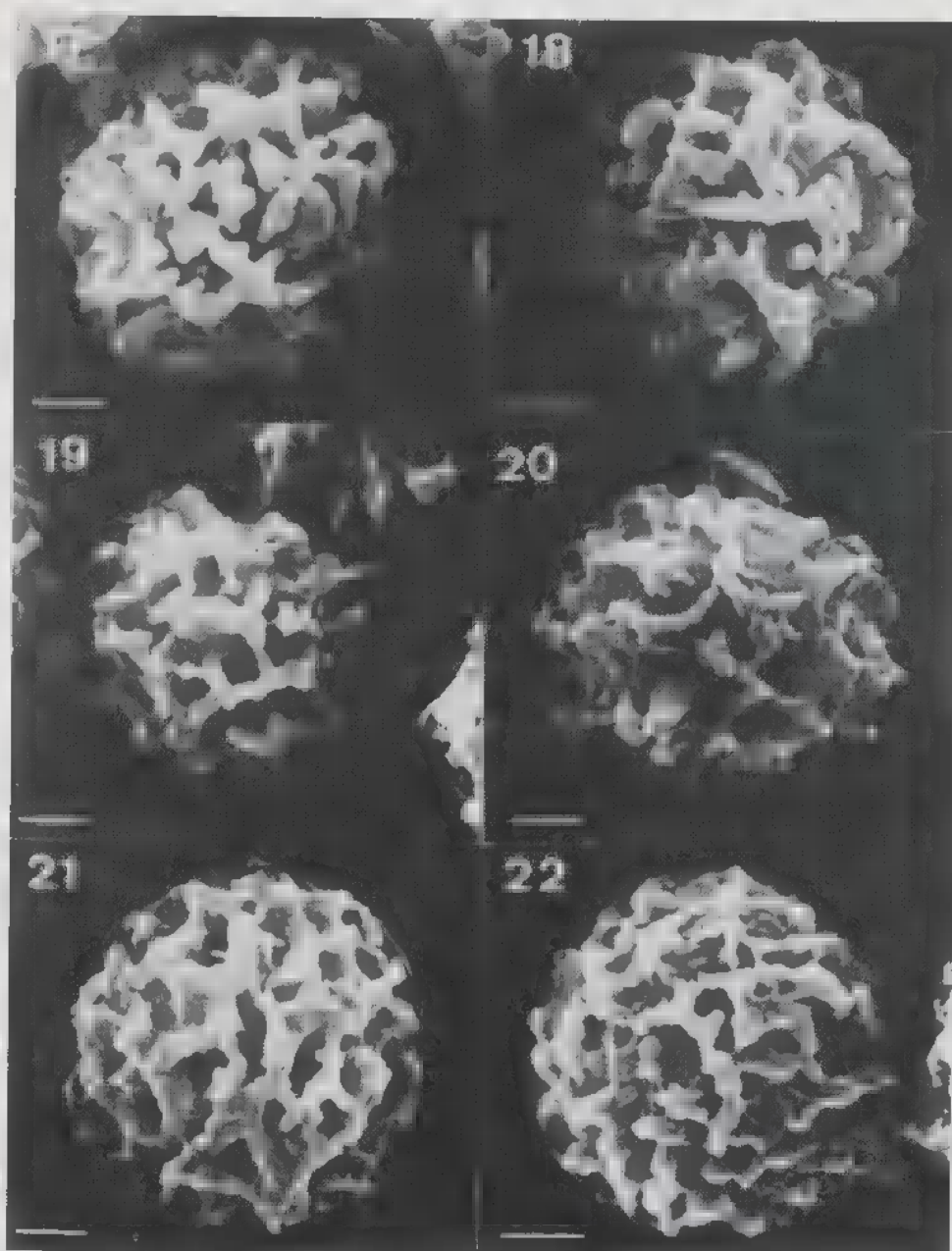
FIGS. 1-3. — *Tulostoma subsquamosum*, S. Ahmad 258 (BAFC), holotype. 1. Basidiocarps showing the tubular mouth. 2, 3. SEM micrographs of spore ornamentation. Scale bars: FIG. 1 = 5 mm, FIGS. 2, 3 = 1 μ m.



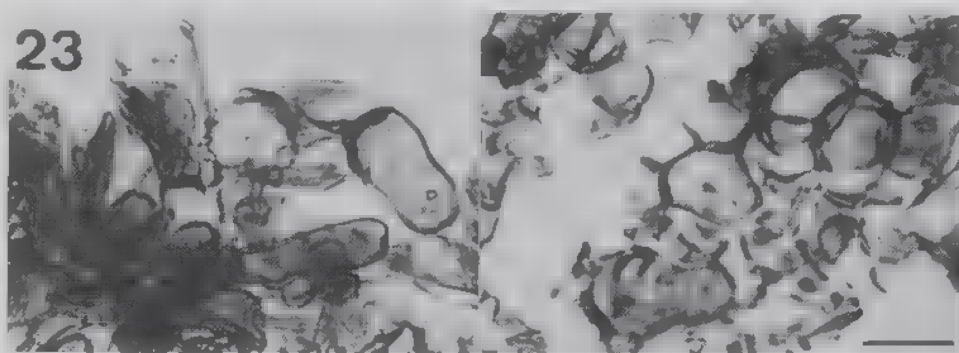
FIGS. 4-12. — *Tulostoma subsquamosum*, S. Ahmad 258 (BAFC), holotype. 4. Original label from S. Ahmad herbarium. 5-7. Light micrographs of capillitium septa. 8-12. Groups of sphaerocysts from the exoperidium. Scale bar = 10 μ m.



FIGS. 13-16. -- *Tulostoma melanocyclus*, K, holotype. 13. Basidiocarps. 14. Detail of a spore-sac showing the tubular mouth and the dark peristome. 15, 16. SEM micrographs of spore ornamentation. Scale bars: FIGS. 13, 14 = 5 mm, FIGS. 15, 16 = 1 μ m.



FIGS. 17-22. — *Tulostoma subsquamosum*, SEM micrographs of spore ornamentation. 17. AH 13826. 18. AH 13827. 19. AH 12449. 20. MA-Fungi 16316. 21. MA-Fungi 20304. 22. BAFC 29454. Scale bars = 1 μ m.



FIGS. 23, 24. — *Tulostoma subsquamosum*, light micrographs of sphaerocysts from the exoperidium.
23. MA-Fungi 20290. 24. BAFC 29454. Scale bar = 10 μ m.